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Developmental Patterns and Cell Lineages of Vermiform Embryos in Dicyemid Mesozoans

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Abstract. Patterns of cell division and cell lineages of the vermiform embryos of dicyemid mesozoans were studied in four species belonging to four genera: *Conocyema polymorpha, Dicyema apalachiensis, Microcyema vespa,* and *Pseudicyema nakaoi.* During early development, the following common features were apparent: (1) the first cell division produces prospective cells that generate the anterior peripheral region of the embryo; (2) the second cell division produces prospective cells that generate the posterior peripheral region plus the internal cells of the embryo; (3) in the lineage of prospective internal cells, several divisions ultimately result in cell death of one of the daughter cells. Early developmental processes are almost identical in the vermiform embryos of all four dicyemid genera. The cell lineages appear to be invariant among embryos and are highly conserved among species. Species-specific differences appear during later stages of embryogenesis. The number of terminal divisions determines variations in peripheral cell numbers among genera and species. Thus, the numbers of peripheral cells are fixed and hence species-specific.

Introduction

All members of the phylum Dicyemida are found in the renal sacs of benthic cephalopod molluscs (Nouvel, 1947; McConnaughey, 1951; Hochberg, 1990). Their bodies consist of the smallest number of cells among multicellular animals (usually 10 to 40) and are organized in a very simple fashion. Although recent studies have revealed that they might not be truly primitive animals deserving the name of mesozoans (Katayama *et al.*, 1995; Kobayashi *et al.*, 1999), they are still one of the most interesting groups of lower invertebrates. Each species is characterized by a fixed number of cells. The somatic cells therefore undergo a limited number of species-specific divisions during embryogenesis. The analysis of embryonic cell lineages in dicyemids is intriguing, since it may provide clues towards an understanding of the simplest patterns of cell differentiation in multicellular animals. A comparative study of cell lineage and developmental processes among related species of dicyemids is also relevant to advance our understanding of morphological evolution in these simple animals.

Dicyemids produce two distinct types of embryos: vermiform embryos from an asexual agamete and infusoriform embryos from fertilized eggs (Furuya *et al.*, 1996). From the standpoint of morphological evolution, the vermiform embryo is the more pertinent target for study because its shape is similar to that of an adult. The cell lineage of vermiform embryos has been fully documented in only two dicyemids, *Dicyema acuticephalum* and *D. japonicum* (Furuya *et al.*, 1994). Among other species, cell lineages have been described only to a limited extent in *Microcyema vespa* and *Pseudicyema truncatum* (Lameere, 1919; McConnaughey, 1938; Schartau, 1940; Nouvel, 1947; Bogomolov, 1970; Lapan and Morowitz, 1975). Details of cell lineage in the phylum as a whole remain to be determined.

In this paper we describe the pattern of cell divisions and cell lineages in the embryogenesis of vermiform embryos in dicyemids belonging to four genera: *Conocyema polymorpha, Dicyema apalachiensis, Microcyema vespa,* and *Pseudicyema nakaoi.* Our data reveal that cell lineages in vermiform embryos are highly conserved among species; but species-characteristic features appear in the later embryogenesis, and these are related to morphological evolution and speciation.

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Materials and Methods

Specimens of *Conocyema polymorpha* van Beneden, 1882, *Dicyema apalachiensis* Short, 1962, *Microcyema vespa* van Beneden, 1882, and *Pseudicyema truncatum* (Whitman, 1883) were examined in the collections of the Department of Invertebrate Zoology, Santa Barbara Museum of Natural History, Santa Barbara, California. *Conocyema polymorpha*, found in *Octopus vulgaris*, was collected by Henri Nouvel in the Mediterranean Sea (Nouvel, 1947). *Microcyema vespa* and *P. truncatum*, found in *Sepia officinalis*, were also collected by Nouvel in the Mediterranean Sea (Nouvel, 1947). *Dicyema apalachiensis*, found in *Octopus joubini*, was collected by Robert B. Short in the Gulf of Mexico off Florida (Short, 1962).

Specimens of *Pseudicyema nakaoi* Furuya, 1999, were prepared for this study. A total of 57 host cuttlefishes, *Sepia esculenta*, were purchased in the western part of Japan. When dicyemids were detected in the kidney of the host cuttlefish, small pieces of renal appendages with attached dicyemids were removed and smeared on slide glasses. The smears were fixed immediately in Bouin's fluid for 24 h and then stored in 70% ethyl alcohol. The fixed smears were stained in Ehrlich's hematoxylin and counterstained in eosin. Stained smears were mounted using Entellan (Merck).

Embryos within the axial cell of parent nematogens were observed with the aid of a light microscope under an oil-immersion objective at a magnification of 2000 diameters. Cells were identified by their position within the embryo, their size, and the intensity of stain taken up by the nucleus and cytoplasm. By careful examination, we were able to identify each swollen nucleus that was about to divide and each metaphase figure in terms of the cell that was about to divide into two daughter cells. Each developing embryo was sketched at three optical depths, and three-dimensional diagrams were reconstructed from these sketches. Measurements and drawings were made with the aid of an ocular micrometer and a drawing tube (Olympus U-DA), respectively. Fully formed embryos consisted at most of 23 cells, and special techniques such as injection of a tracer and videoscopy were not required for determination of the cell lineage. Early divisions of the vermiform embryos examined in this study were the same as those of *Dicyema acuticephalum* and *D. japonicum* (see Furuya *et al.*, 1994). The terminology of cells used by Furuya *et al.* (1994) was adopted in designating the cells in the present paper.

Results

In the Dicyemida, two adult forms, the nematogen and the rhombogen, develop asexually from an agamete (axoblast) through a vermiform embryo within the axial cell of parent nematogens (Fig. 1).

Microcyema vespa

Agamete diameter is about 6 μm. The first division is meridional and unequal, producing two daughter cells, A and B. Cell B becomes the mother cell of the peripheral cells of the embryo's head. The second division involves only cell B. This division is occasionally skipped. It is extremely unequal, producing two daughter cells that are quite different in size. The smaller of these two cells ultimately degenerates without contributing to embryogenesis. The third division, involving cell A, is latitudinal and equal, producing two daughter cells, 2A and 2a. Cell 2A is the mother cell of peripheral cells in the tail, and cell 2a is the prospective axial cell. In the 2a lineage, extremely unequal divisions occur at around the 5- and 7-cell stages. The resultant much smaller daughter cells remain attached to the larger daughter cells until they ultimately degenerate without contributing to embryogenesis. The fourth division, involving cell 2B, is meridional and equal, producing two daughter cells, 3B and $\underline{3B}$. At this 4-cell stage, two pairs of cells, 2A-2a and 3B-$\underline{3B}$, are arranged crosswise with respect to one another. The furrow of the fourth division coincides with the plane of bilateral symmetry of the embryo. The pattern of division and the cell lineage are the same for the descendants of cell 3B and those of cell $\underline{3B}$. The fifth division, involving cell 2A, is latitudinal and equal; resulting in the 5-cell embryo. The plane of cell division coincides with the plane of bilateral symmetry, and it separates the right cell $\underline{3A}$ from the left cell 3A. These cells do not divide further but become the two peripheral cells of the tail region, known as the uropolar cells (Figs. 2a-c, 3a).

The pattern of cell division beyond the 5-cell stage changes from spiral to bilateral. After the 5-cell stage, divisions occur not one by one but in pairs, and the divisions become almost synchronized. Subsequent developmental stages thus proceed with odd numbers of cells, yielding, for example, a 7-cell embryo, and so on.

The sixth division is extremely unequal. Both the 3B and $\underline{3B}$ cells divide, and they produce a pair of large cells and a pair of much smaller daughter cells. The smaller cells degenerate and eventually disappear. At around the 5-cell stage, cell 2a, the prospective axial cell, undergoes an extremely unequal division. The resultant smaller cell degenerates and eventually disappears. The seventh division is equal, and results in the 7-cell embryo. Cells 4B and $\underline{4B}$ divide and produce two pairs of daughter cells, $5B^1$ and $5B^2$ plus $\underline{5B^1}$ and $\underline{5B^2}$, respectively. The future anterior-posterior axis of the embryo corresponds almost exactly to the $5B^1$-3A axis at the 7-cell stage. About the 7-cell stage, cell 3a, the prospective axial cell, undergoes an extremely unequal division. The resulting smaller cells degenerate and eventually disappear.

After the seventh division, the order of division is not necessarily identical among developing embryos. The

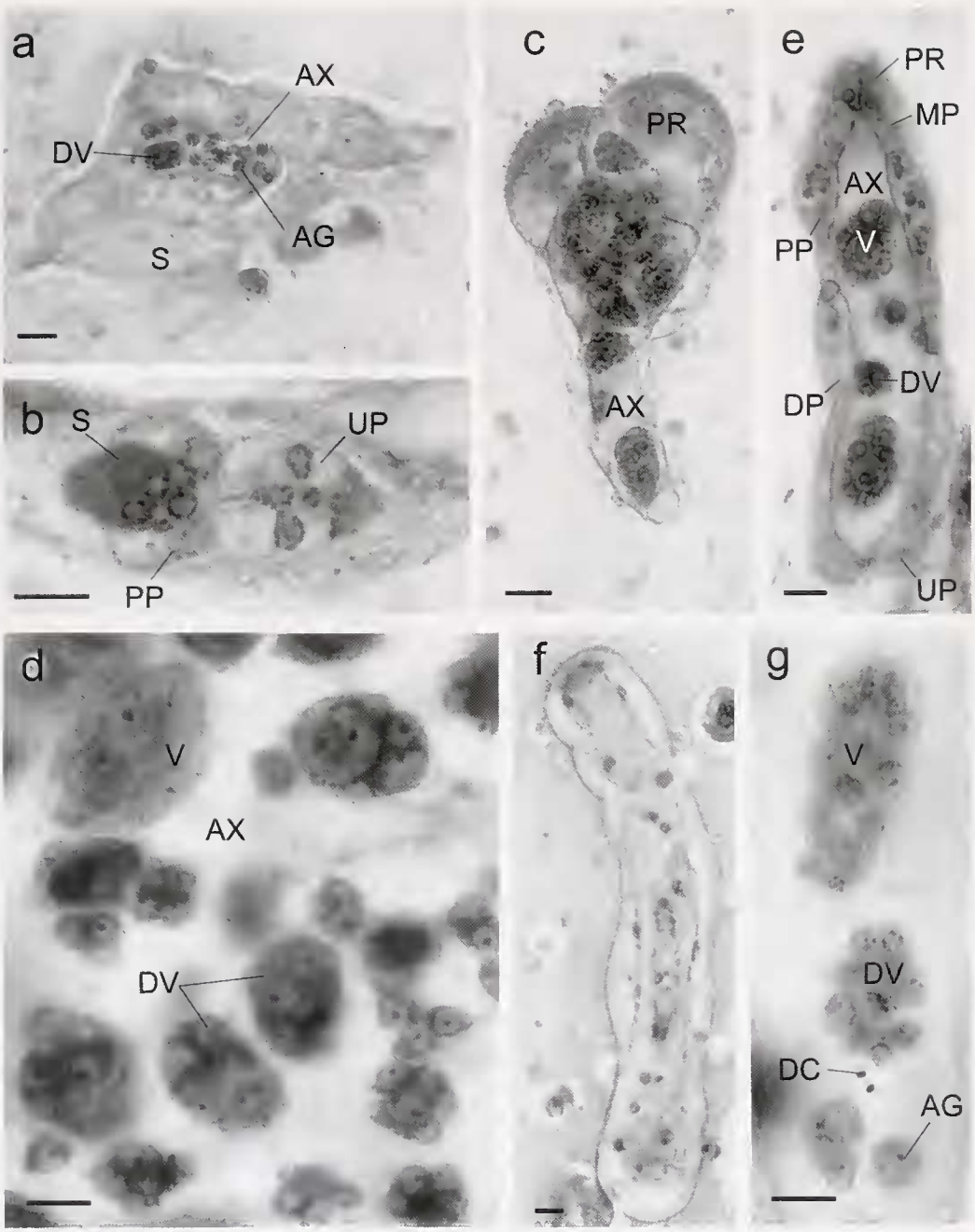

Figure 1. Light micrographs of nematogens in four species of dicyemids. Scale bars represent 10 μm. Abbreviations: AG, agamete; AX, axial cell; DC, degenerating cell; DP, diapolar cell; DV, developing vermiform embryo; MP, metapolar cell; PP, parapolar cell; PR; propolar cell; S, syncytium; UP, uropolar cell; V, vermiform embryo. *Microcyema vespa*: (**a**) whole body of a young individual; (**b**) a vermiform embryo in the axial cell of the nematogen. *Conocyema polymorpha*: (**c**) whole body of a nematogen; (**d**) developing vermiform embryos in the axial cell of the nematogen. *Dicyema apalachiensis*: (**e**) whole body of the nematogen. *Pseudicyema nakaoi*: (**f**) whole body of a nematogen; (**g**) developing vermiform embryos in the axial cell of the nematogen.

$5B^1$ cell pair divides equally and produces two pairs of daughter cells, $6B^{11}$ and $6B^{12}$ plus $\underline{6B^{11}}$ and $\underline{6B^{12}}$. The $5B^2$ cell pair divides equally and produces two pairs of daughter cells, $6B^{21}$ and $6B^{22}$ plus $\underline{6B^{21}}$ and $\underline{6B^{22}}$. Neither pair divides further, and they form the anterior part of the embryo (Fig. 2a, b). The $6B^{22}$ cell pair develops into the parapolar cells, while the $6B^{11}$, $6B^{12}$, and $6B^{21}$ cell pairs eventually form a syncytium, which is more

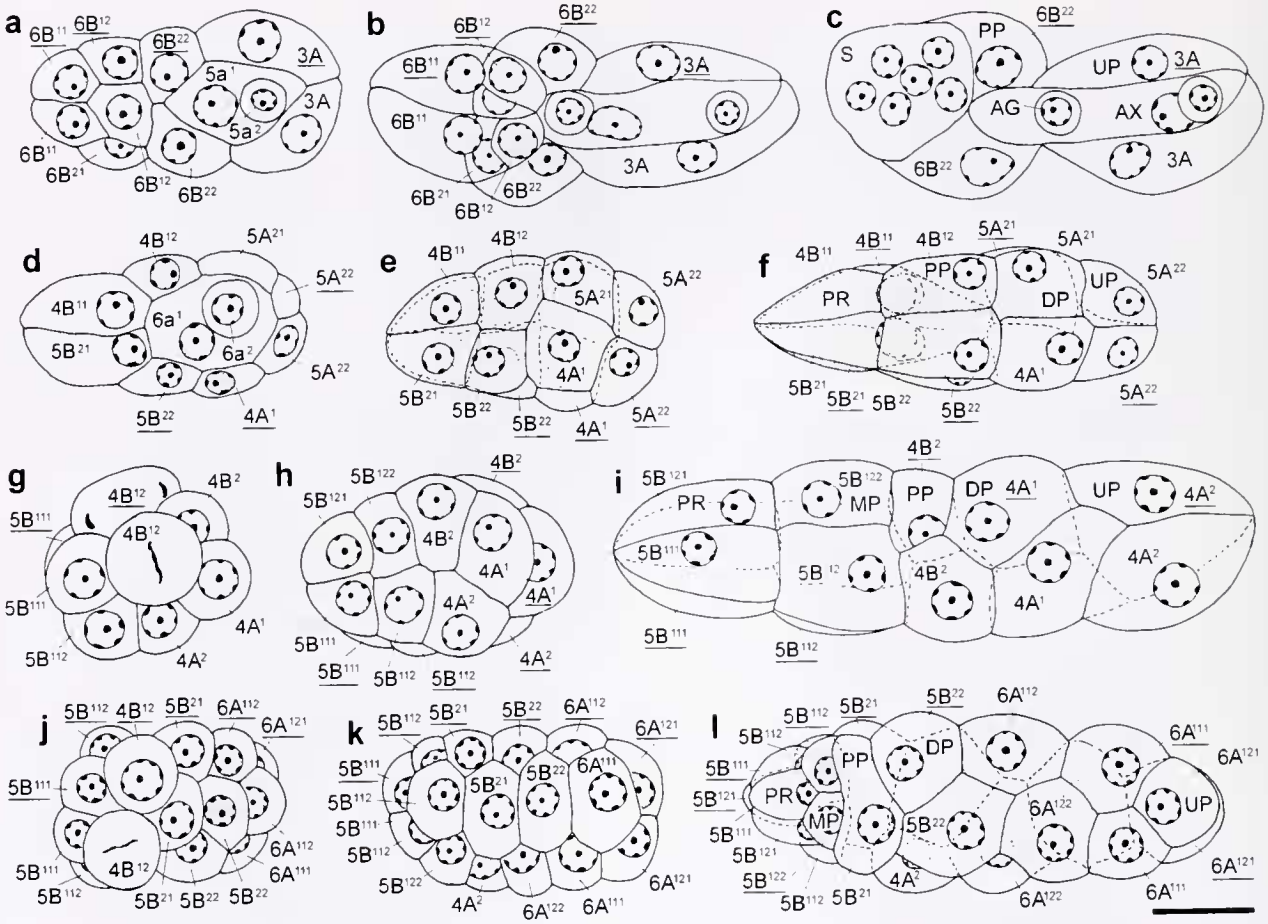

Figure 2. The late-stage vermiform embryos of four species of dicyemids. Scale bar represents 10 μm. Cilia are omitted. See text for explanations of cell division notations. Other abbreviations: AG, agamete; AX, axial cell; DP, diapolar cell; MP, metapolar cell; PR, propolar cell; PP, parapolar cell; S, syncytium; UP, uropolar cell. *Microcyema vespa*: (**a**) a late-stage embryo (sagittal optical section)—note an agamete ($5a^2$) in the cytoplasm of an axial cell ($5a^1$); (**b**) a late-stage embryo (sagittal optical section); (**c**) formed embryo (sagittal optical section)—pairs of $6B^{11}$, $6B^{12}$, and $6B^{21}$ cells form a syncytium (S) that is more conspicuously stained with hematoxylin. *Conocyema polymorpha*: (**d**) a late-stage embryo (sagittal optical section)—note an agamete ($6a^2$) incorporated in the cytoplasm of an axial cell ($6a^1$); (**e**) a late-stage embryo (lateral view); (**f**) formed embryo (lateral view)—pairs of $4B^{11}$ and $5B^{21}$ cells form propolar cells (PR) that are more conspicuously stained with hematoxylin. *Dicyema apalachiensis*: (**g**) 13-cell stage—note an anaphase figure of $4B^{12}$ cell and a metaphase figure of $4B^{12}$ cell; (**h**) 15-cell stage—the $5B^{111}$ and $5B^{121}$ pairs form the propolar cells (PR), while the $5B^{112}$ and $5B^{122}$ pairs form another type of polar cell, the metapolar cell (MP); (**I**) formed embryo (lateral view)—propolar cells and metapolar cells are more conspicuously stained with hematoxylin. *Pseudicyema nakaoi*: (**j**) 22-cell stage—note a metaphase figure in $4B^{12}$ cell—the plane of this division, in contrast to the divisions of $4B^{12}$ pair (Fig. 2g), are oblique to the anterior-posterior axis, and as the result, cells of the propolar tier alternate with cells of the metapolar tier (see Fig. 2k, l); (**k**) a late-stage embryo (lateral view); (**l**) formed embryo (lateral view)—propolar cells and metapolar cells are more conspicuously stained with hematoxylin.

conspicuously stained with hematoxylin than the other cells (Fig. 1b). The two parapolar cells cover more than half of the syncytium (Fig. 2c). As peripheral cells are formed, the prospective axial cell, 4a, divides unequally. The large anterior cell, $5a^1$, undergoes no further divisions and becomes the axial cell, while the smaller posterior cell, $5a^2$, is incorporated into the axial cell and becomes an agamete (Fig. 2a). At this stage, cilia are first evident on the peripheral cells. Cilia on the external surface of the syncytium are directed anteriorly and are more densely distributed than on other peripheral cells.

The fully formed embryo consists of three types of cells: peripheral cells, one syncytial cell, and an axial cell, which contains two agametes (Figs. 1b, 2c). The

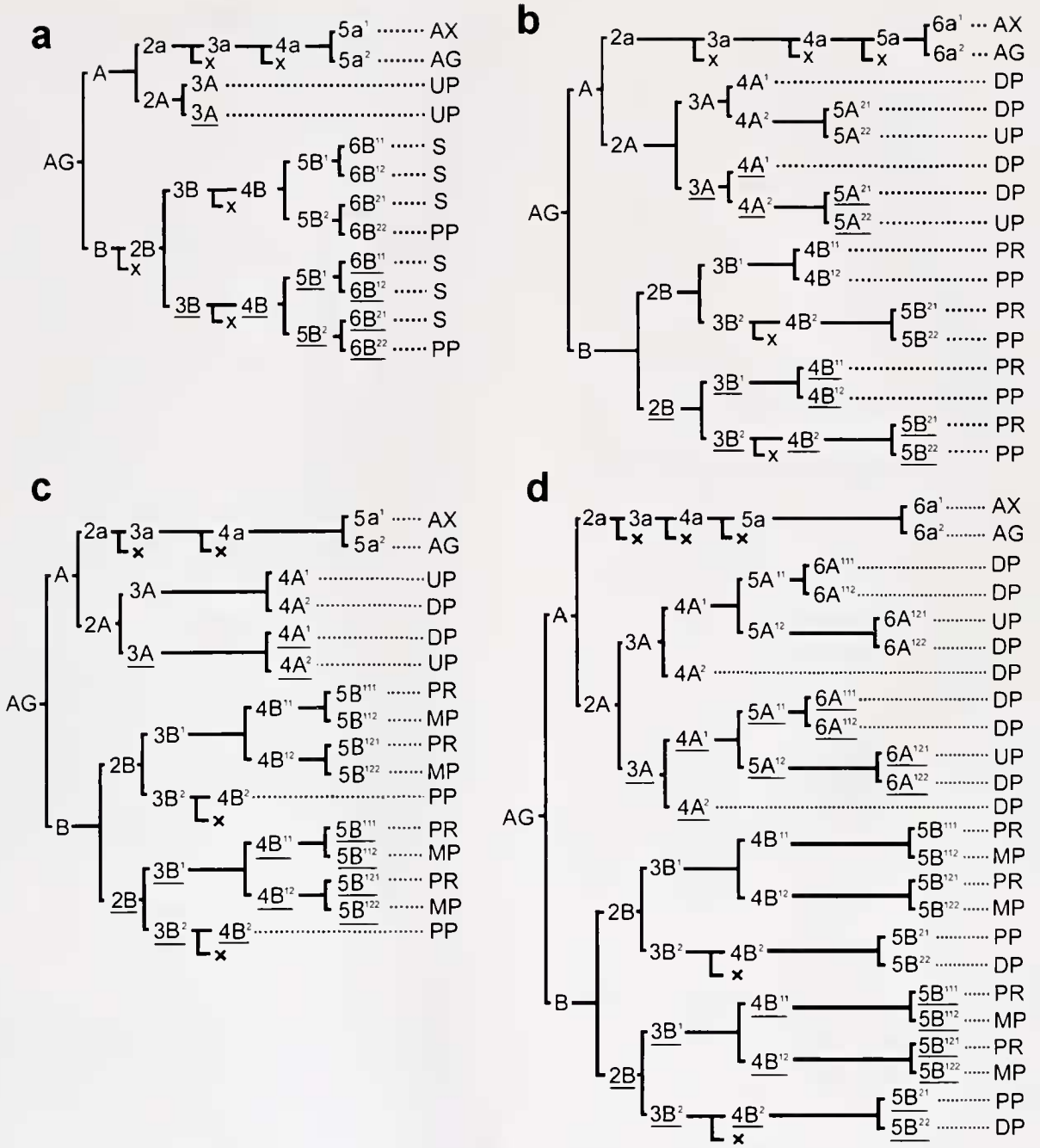

Figure 3. Cell lineages of vermiform embryos. A cross (x) indicates that a cell, formed as the result of an unequal division, degenerates and does not contribute to the formation of the embryo. See text for explanations of cell division notations. Other abbreviations: AG, agamete; AX, axial cell; DP, diapolar cell; MP, metapolar cell; PP, parapolar cell; PR, propolar cell; S, syncytium; UP, uropolar cell. (**a**) *Microcyema vespa*; (**b**) *Conocyema polymorpha*; (**c**) *Dicyema apalachiensis*; (**d**) *Pseudicyema nakaoi*.

peripheral cells are composed of two parapolar cells and two uropolar cells. The swollen cephalic head region consists of a calotte and two parapolar cells. The calotte is a syncytium. The trunk is composed of two uropolar cells. Further development involves growth and enlargement of the syncytium and branching of the axial cell (Fig. 1a). Total length, excluding cilia, of the fully formed vermiform embryo is about 50 μm, and the width is about 20 μm. The cell lineage of the vermiform embryo is summarized in Figure 3a. No variations in cell lineage were found in more than 50 embryos examined.

Conocyema polymorpha

Agamete diameter is about 7 μm. The first division is meridional and unequal, producing two daughter cells that are slightly different in size, A and B. The larger cell B becomes the mother cell of the peripheral cells of the embryo's head (propolar and parapolar cells). The second division involves only the smaller cell A. This division is latitudinal and equal, producing two daughter cells, 2A and 2a. Cell 2A is the mother cell of the peripheral cells of the posterior trunk and tail, and cell 2a is the prospective axial cell (Fig. 3b). In the 2a lineage, extremely unequal divisions occur at around the 5-, 11-, and 13-cell stages (Fig. 3b), and the resultant much smaller daughter cells remain attached to the larger daughter cells until they ultimately degenerate without contributing to embryogenesis. The third division, involving cell B, is meridional and equal, producing two daughter cells, 2B and 2B. At this 4-cell stage, two pairs of cells, 2A-2a and 2B-2B, are arranged crosswise with respect to one another. The furrow of the third division coincides with the plane of bilateral symmetry of the embryo. The pattern of division and the cell lineage are the same for the descendants of cell 2B and those of cell 2B (Fig. 3b). The fourth division, involving cell 2A, is meridional and equal, resulting in the 5-cell embryo. The plane of cell division again coincides with the plane of bilateral symmetry, and it separates the right cell 3A from the left cell 3A. The pattern of cell division and the cell lineage are the same for descendants of cell 3A and for those of cell 3A (Fig. 3b).

The pattern of cell division beyond the 5-cell stage changes from spiral to bilateral. Beyond the 5-cell stage, divisions occur not one by one but in pairs, and they become almost synchronized. Subsequent developmental stages thus proceed with odd numbers of cells.

At around the 5-cell stage, cell 2a, the prospective axial cell, undergoes an extremely unequal division. The resultant smaller cell degenerates and finally disappears. The fifth cell division is equal and results in the 7-cell embryo. Thus, cells 2B and 2B divide and produce two pairs of daughter cells, $3B^1$ and $3B^2$ plus $\underline{3B^1}$ and $\underline{3B^2}$, respectively. The future anterior-posterior axis of the embryo corresponds almost exactly to the $3B^1$-3A axis at the 7-cell stage. The sixth division is slightly unequal. Cells 3A and 3A divide into two pairs of daughter cells, $4A^1$ and $4A^2$ plus $\underline{4A^1}$ and $\underline{4A^2}$. Cells $4A^2$ and $\underline{4A^2}$ are the smallest cells at this stage. In addition to the 2a lineage, cells $3B^2$ and $\underline{3B^2}$ undergo unequal divisions, each generating a pair of cells, one large and one much smaller. The smaller cells degenerate and finally disappear, although they remain in place on the developing embryo until later stages.

The $3B^1$ pair divide equally into $4B^{11}$ and $4B^{12}$ pairs. These cells undergo no further divisions. The $4B^{11}$ pair become the propolar cells, and the $4B^{12}$ pair become the parapolar cells (Figs. 2e, f). The cell in the 2a lineage (cell 3a) is incorporated into the inside of the embryo, and the $3B^1$ pair divide and rearrange their descendants. At around the 11-cell stage, the 3a cell undergoes an extremely unequal division.

The 13-cell stage is achieved by equal divisions of cells $4A^2$ and $\underline{4A^2}$. The resultant $5A^{21}$ and $5A^{22}$ pairs undergo no further divisions and become diapolar cells and uropolar cells, respectively. Soon after these divisions, the $4B^2$ pair divide equally into two pairs of daughter cells, $5B^{21}$ and $5B^{22}$ plus $\underline{5B^{21}}$ and $\underline{5B^{22}}$. The $5B^{21}$ and $5B^{22}$ pair undergo no further divisions and become the propolar cells and parapolar cells, respectively (Fig. 2e, f). At around the 13-cell stage, the prospective axial cell 4a undergoes an extremely unequal division.

At the final stage of embryogenesis, the prospective axial cell, 5a, divides unequally. The large anterior cell, $6a^1$, undergoes no further divisions and becomes the axial cell, while the smaller posterior cell, $6a^2$, is incorporated into the axial cell and becomes an agamete (Fig. 2d).

The fully formed vermiform embryo of *Conocyema polymorpha* consists of 14 peripheral cells and one axial cell, which contains one or two agametes (Fig. 2f). The head peripheral cells are composed of four propolar cells and parapolar cells. The propolar cells have short, dense cilia and form the calotte, which is more conspicuously stained with hematoxylin than the other cells. Four diapolar cells make up the trunk peripheral cells. The caudal peripheral cells are uropolar cells. The length, excluding cilia, of the fully formed embryo is about 25 μm, and the width is about 10 μm. The cell lineage of the vermiform embryo is summarized in Figure 3b. No variations in cell lineage were found in more than 80 embryos examined.

Dicyema apalachiensis

Agamete diameter is about 5.5 μm. The first division is meridional and equal, producing two daughter cells of equal size. The subsequent patterns of development and cell lineage up to the 7-cell stage are the same as those described for *Conocyema polymorpha*.

At the 7-cell stage, cells $3B^2$ and $\underline{3B^2}$ undergo unequal divisions, each generating a pair of cells, one large and one much smaller cell. The 9-cell stage is achieved by unequal divisions of cells $3B^1$ and $\underline{3B^1}$. The resultant small cells, $4B^{11}$ and $\underline{4B^{11}}$, divide again into two pairs of daughter cells, $5B^{111}$ and $5B^{112}$ plus $\underline{5B^{111}}$ and $\underline{5B^{112}}$, in the anterior part of the embryo (Fig. 2g-i). Almost simultaneously, the resultant large cells, $4B^{12}$ and $\underline{4B^{12}}$, divide again into two pairs of daughter cells, $5B^{121}$ and $5B^{122}$ plus $\underline{5B^{121}}$ and $\underline{5B^{122}}$, in the anterior part of the embryo (Fig. 2h, i). These cells undergo no further divisions, and the $5B^{111}$ and $5B^{121}$ pairs become the propolar cells, while the $5B^{112}$ and $5B^{122}$ pairs become the metapolar cells. The cell in the 2a lineage (cell 4a) is

incorporated into the inside of the embryo, and the other cells, $4B^{11}$ and $\underline{4B^{11}}$, divide and rearrange their descendants.

At the 9-cell stage, cells 3A and $\underline{3A}$ divide equally into two pairs of daughter cells, $4A^1$ and $4A^2$ plus $\underline{4A^1}$ and $\underline{4A^2}$. They do not divide further; the $4A^1$ pair become the uropolar cells, and the $4A^2$ pair become the diapolar cells (Fig. 3c). At the final stage of embryogenesis, the prospective axial cell, 4a, divides unequally. The large anterior cell, $5a^1$, becomes the axial cell, and the smaller posterior cell, $5a^2$, is incorporated into the axial cell and becomes an agamete.

The vermiform embryo of *Dicyema apalachiensis* consists of 14 peripheral cells and one axial cell, which contains one or two agametes. The peripheral cells of the head region are composed of four propolar cells, four metapolar cells, and two parapolar cells. The propolar and metapolar cells have short, dense cilia and form the calotte. Two diapolar cells make up the trunk peripheral cells. Two caudal peripheral cells are uropolar cells. The length, excluding cilia, of the fully formed embryo is about 30 μm, and the width is about 10 μm. The cell lineage of the vermiform embryo is summarized in Figure 3c. No variations in cell lineage were found in more than 50 embryos examined.

Pseudicyema nakaoi

Agamete diameter is about 6.5 μm. The first division is equal, producing two daughter cells of equal size. The subsequent patterns of development and cell lineage up to the 9-cell stage are the same as those described for *Dicyema apalachiensis* (see Fig. 3c).

At the 9-cell stage, cells $3B^2$ and $\underline{3B^2}$ undergo extremely unequal divisions, each generating a pair of cells, one large and one much smaller cell. The smaller cells resulting from this division degenerate and finally disappear. In the 2a line, unequal divisions occur at around the 5-, 9-, and 11-cell stages (Fig. 3d). The pattern of development and cell lineages up to the 9-cell stage are the same in both *P. truncatum* and *P. nakaoi*. Further development was not studied in *P. truncatum*, because adequate material was not available.

After the unequal divisions of the $3B^2$ pair, cell pairs $4A^1$ and $3B^1$ undergo equal divisions almost simultaneously and produce two pairs of daughter cells, $5A^{11}$ and $5A^{12}$ plus $4B^{11}$ and $4B^{12}$, to form the 13-cell-stage embryo. At around the 13-cell stage, the prospective axial cell, 4a, undergoes an unequal cell division. The $5A^{11}$ pair divide equally and produce two pairs of daughter cells, $6A^{111}$ and $6A^{112}$ plus $\underline{6A^{111}}$ and $\underline{6A^{112}}$. The plane of this division is parallel to the anterior-posterior axis, in contrast to the previous division, which occurs parallel to the perpendicular axis. As a result, cells $6A^{111}$ and $\underline{6A^{111}}$ are situated on the left and right sides of the embryo, respectively.

At the 15-cell stage, cell pairs $4B^2$ and $5A^{12}$ undergo equal divisions almost simultaneously, and produce two pairs of daughter cells, $5B^{21}$ and $5B^{22}$ plus $6A^{121}$ and $6A^{122}$, to form the 19-cell-stage embryo. These pairs undergo no further divisions. Cell pair $5B^{21}$ become the parapolar cells, and the $5B^{22}$ pair become the anterior diapolar cells (Fig. 3d). Cell pair $6A^{121}$ become the uropolar cells, and the $6A^{122}$ pair become the posterior diapolar cells (Fig. 3d).

At the 19-cell stage, the $4B^{11}$ and $4B^{12}$ pairs divide equally into two pairs of daughter cells, $5B^{111}$ and $5B^{112}$ plus $5B^{121}$ and $5B^{122}$, in the anterior part of the embryo (Fig. 2k, l). These divisions proceed cell by cell. The daughter cells undergo no further divisions, and the $5B^{111}$ and $5B^{121}$ pairs become the propolar cells, while the $5B^{112}$ and $5B^{122}$ pairs become the metapolar cells (Fig. 2j). The planes of these divisions, in contrast to the previous division, are oblique to the anterior-posterior axis. As the result, cells of the propolar tier alternate with cells of the metapolar tier (Fig. 2k, l).

At the final stage of embryogenesis, the prospective axial cell, 5a, divides unequally. The large anterior cell, $6a^1$, becomes the axial cell, and the smaller posterior cell, $6a^2$, is incorporated into the axial cell and becomes an agamete.

The fully formed vermiform embryo of *P. nakaoi* consists of 22 peripheral cells and one axial cell, which contains one agamete. The peripheral cells of the head region are composed of four propolar cells, four metapolar cells, and two parapolar cells. The propolar and metapolar cells have dense short cilia and form the calotte. Ten diapolar cells make up the trunk peripheral cells. Two caudal peripheral cells are uropolar cells. The body length, excluding cilia, of the fully formed embryo is about 70 μm, and the body width is about 16 μm. The cell lineage of the vermiform embryo is summarized in Figure 3d. No variations in cell lineage were found in more than 50 embryos examined.

Discussion

Patterns of development of the vermiform embryos of four species of dicyemids belonging to four genera, namely *Microcyema vespa*, *Conocyema polymorpha*, *Dicyema apalachiensis*, and *Pseudicyema nakaoi*, were studied in detail. In the embryogenesis of each species, cell divisions proceed without variation and result in fully formed embryos with a definite number and arrangement of cells. The process of development of vermiform embryos is very simple and seems to be programmed similarly to that of infusoriform embryos and infusorigens (Furuya *et al.*, 1992b, 1993, 1995). Seven different cell lineages including those of two previously described species, *D. acuticephalum* and *D. japonicum* (Figs. 3, 4; see also Furuya *et al.*, 1994), could be compared. Early developmental processes up to the 7-cell stage are almost identical in vermiform embryos examined in this study and those of *D. acuticephalum* and *D. japonicum* (Figs. 5, 6).

Our results are different from earlier reports with respect to the timing of cell-fate specification (Lameere, 1919;

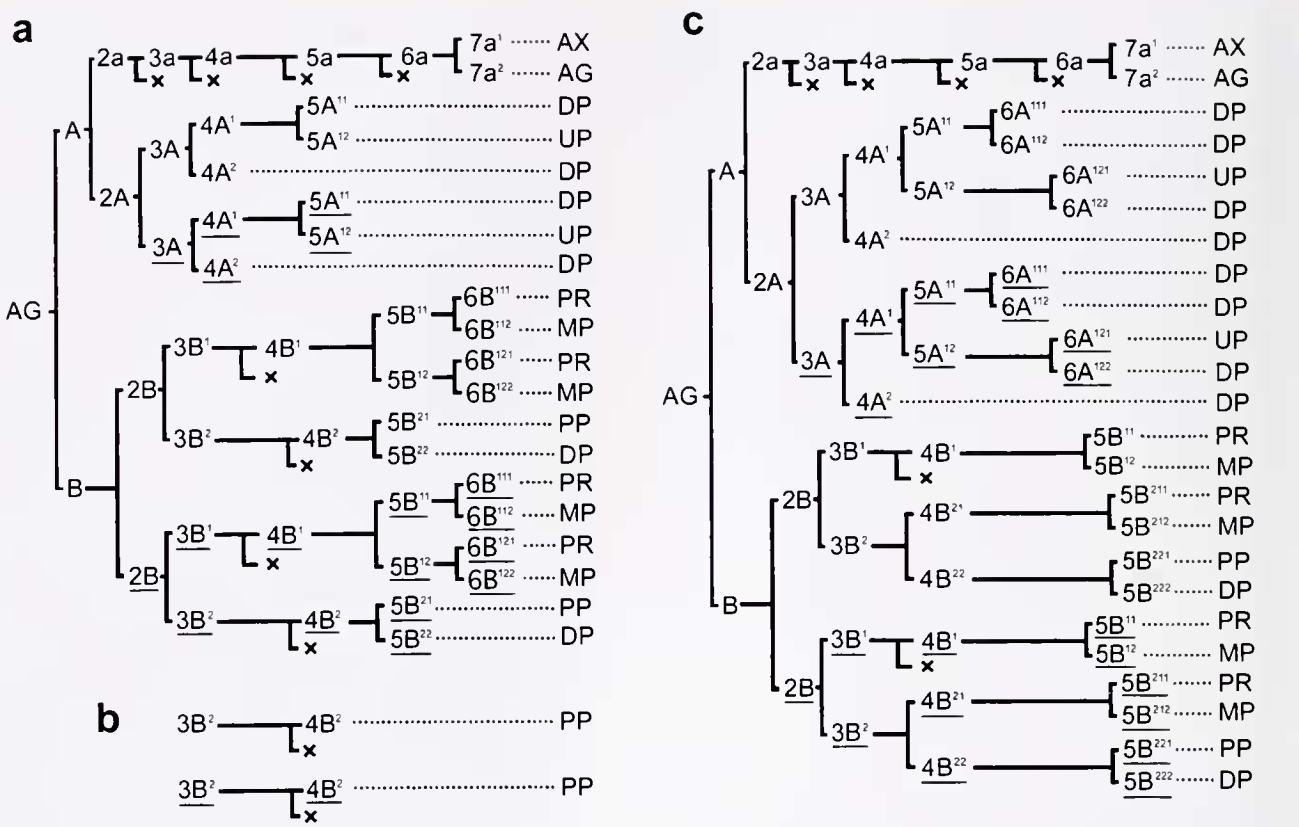

Figure 4. Cell lineages of vermiform embryos in *Dicyema acuticephalum* and *D. japonicum* (modified from Furuya *et al.*, 1994). A cross (x) indicates that a cell resulting from an unequal division degenerates and does not contribute to the formation of the embryo. Abbreviations: AG, agamete; AX, axial cell; DP, diapolar cell; MP, metapolar cell; PP, parapolar cell; PR, propolar cell; UP, uropolar cell. (**a**) *D. acuticephalum* with 18 peripheral cells; (**b**) *D. acuticephalum* with 16 peripheral cells; (**c**) *D. japonicum*.

Gersch, 1938; McConnaughey, 1951). According to Lameere (1919), in *M. vespa* and *P. truncatum* the first division is unequal, and as a result two daughter cells of different sizes are produced. One of the daughter cells (usually the larger one) is described as a prospective axial cell, and the other is regarded as the mother cell of the peripheral cells. However, in all species examined, we found that the prospective axial cell was produced at the second division, not at the first division. Gersch (1938) and McConnaughey (1951) also claimed that the prospective axial cell is produced at the first division in *D. typus*, *D. balamuthi*, *Dicyemennea abelis*, and *Dicyemennea californica*. Although the possibility that two types of first division exist cannot be excluded in this study, the results of those early observations remain to be confirmed.

Early developmental pattern and cell lineage

Comparisons of developmental processes and cell lineages among various species of dicyemids reveal conservative features in the early development. Although dicyemids from different host species and geographically different distributions were compared, the developmental processes and cell lineages are almost identical from an agamete to the 7-cell stage (Fig. 5). Cell-fate segregation appears in the very early stages of embryogenesis. Three types of prospective cells that form the body of embryos, such as the agamete, the axial cell, and the peripheral cells, can be identified as early as the 3-cell stage. In the development of vermiform embryos, cell fates may be initially segregated. This conserved feature among species may represent the basic plan in forming bodies of vermiform embryos (Fig. 6). These features in cell lineage suggest that the early developmental processes have persisted through the evolution of dicyemids. Vermiform embryos develop in the confined space of an axial cell located within the parent nematogen. This peculiar habitat thus may constrain the developmental process, as well as limit the size and number of cells that compose the body. As a result, development may appear to be conserved.

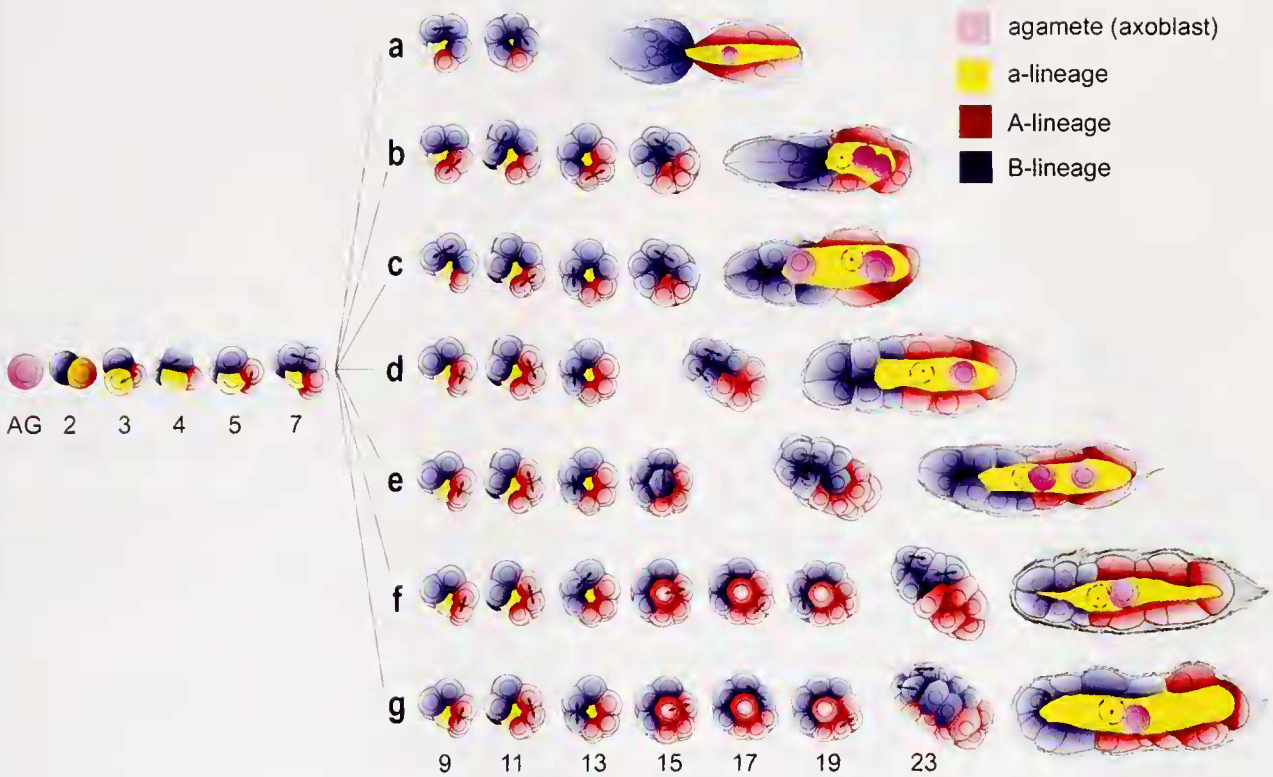

Figure 5. Developmental processes of vermiform embryos in several species of dicyemids. The developmental patterns and cell lineages from the agamete (AG) to 7-cell stage are identical among the species. The numerals in the bottom row represent cell number stages in the development. Arrows in the developing embryos indicate daughter cells that were produced by the proceeding division. (**a**) *Microcyema vespa*; (**b**) *Conocyema polymorpha*; (**c**) *Dicyema apalachiensis*; (**d**) *D. acuticephalum* with 16 peripheral cells; (**e**) *D. acuticephalum* with 18 peripheral cells; (**f**) *D. japonicum*; (**g**) *Pseudicyema nakaoi*.

Variations of terminal divisions in cell lineages

Species-specific patterns of development and cell lineages appear in the later stages of embryogenesis. The most striking difference is seen in terminal divisions in the cell lineage that give rise to variations in peripheral cell numbers. For instance, species-specific differences in the peripheral cell number between *Dicyema acuticephalum* and *D. japonicum* can be attributed to the number of divisions of the $4A^1$ pair (Fig. 4; Furuya *et al.*, 1994). In other species, additional terminal divisions occur toward the end of the establishment of another cell lineage as well. The various numbers of terminal divisions, which are genetically determined, clearly play a significant role in the morphogenesis of vermiform embryos and may be correlated with speciation in the dicyemids.

In most species of dicyemids, vermiform embryos have a constant number of peripheral cells. However, some species of dicyemids, such as *Dicyema acuticephalum, D. bilobum, D. benthoctopi, D. erythrum, D. lycidoceum*, and *D. rhadinum*, have a variable number of peripheral cells (Nouvel, 1947; Couch and Short, 1964; Hochberg and Short, 1970; Furuya *et al.*, 1992a; 1994; Furuya, 1999). Such intraspecific variation in peripheral cell numbers could be attributed to minor differences in numbers of terminal divisions in certain cell lineages (compare Fig. 4a and b).

In the developmental patterns of vermiform embryos, the cell lineages do not vary, and the terminal divisions usually occur bilaterally. Thus, several even numbers of peripheral cells are formed as the result of a pair of terminal divisions in both the 2A- and B-cell lineages. In species that have a variable number of peripheral cells, such as *Dicyema erythrum, D. lycidoceum*, and *D. rhadinum*, some peaks are evident in even numbers of peripheral cells (see tables in Furuya, 1999). The number of terminal divisions may not be strictly programmed in these exceptional species.

Later development and larval morphology

In the evolution of dicyemids, various types of vermiform embryos must have been produced as deviations from a common developmental pattern. Unusual species, such as *Microcyema vespa* and *Conocyema polymorpha*, not only differ morphologically from other dicyemids but are distinct

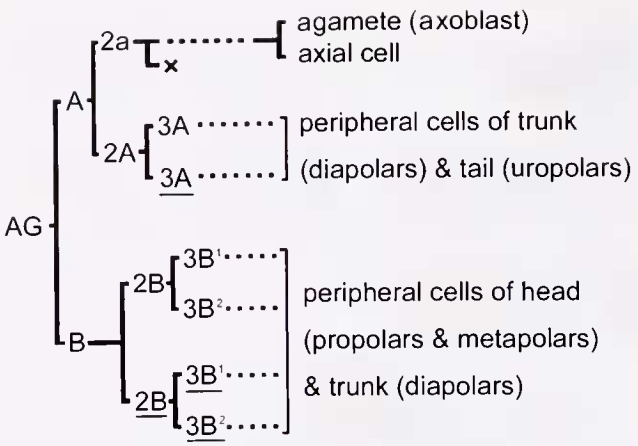

Figure 6. A common cell lineage in all the vermiform embryos examined. At the first division, an agamete (AG) divides to produce two daughter cells, A and B. Cell A divides into two daughter cells. Cell 2a is a mother cell for both an axial cell and agamete. Descendants of cell 2A form the peripheral cells of both trunk and tail. Descendants of cell B form the peripheral cells of both the head and anterior trunk. A cross (x) indicates that a cell formed by unequal division degenerates and does not contribute to the formation of the embryo.

in the later stages of development. As shown in the cladogram (Fig. 7), these two species of dicyemids are clearly distinct and separate when compared with the clade composed of the genera *Dicyema* and *Pseudicyema*. Some changes that occur in cell lineages certainly are reflected in morphological features.

The genus *Pseudicyema*, as diagnosed by Nouvel (1933), is morphologically very similar to *Dicyema*. As a result, it occasionally has been treated as a subgenus of *Dicyema* (Hochberg, 1990). The difference between *Pseudicyema* and *Dicyema* depends on whether cells of the propolar tier are alternate or opposite with respect to the cells in the metapolar tier. The developmental processes in these genera are different only in the terminal cell lineage and the pattern of cell divisions at the final stage of embryogenesis. On the basis of cell lineages, differences between *Dicyema* and *Pseudicyema* are within the range of inter-species differences in *Dicyema*, as shown in the cladogram. However, as far as calotte configuration and the process of calotte formation are concerned, *Dicyema* and *Pseudicyema* can be clearly identified as separate groups. Although cell lineage is an important character, it may not necessarily help to determine the definition of genera. Detailed comparative studies on cell lineages and organization of infusoriform embryos are also indispensable in separating dicyemid taxa.

In recent years, it has been argued that the evolution of morphological features requires alterations in developmental processes. In dicyemids, the cell lineage of *Microcyema vespa* is closer to a conservative lineage than in other genera in the phylum, but vermiform embryos of *M. vespa* show a distinctive form not seen in other genera. It is possible that in *M. vespa* the developmental process may be truncated, resulting in a simple cell lineage and a body organization with a very small number of peripheral cells. However, changes in cell lineage may not always contribute to morphological characters. For example, there are some differences in the later cell lineage between *Dicyema acuticephalum* and *D. apalachiensis*, but these dicyemids are very similar in general body shape.

Cell death

McConnaughey (1951) described chromatin elimination from the prospective axial cell. In *Dicyema acuticephalum* and *D. japonicum*, what appears to be a mass of eliminated chromatin is actually a small cell that is produced as the result of an extremely unequal division (Furuya *et al.*, 1994). In *Pseudicyema truncatum* and *Microcyema vespa*, Lameere (1919) noted that the prospective axial cell underwent an unequal division and that the smaller daughter cell itself divided once or twice to produce two or four small

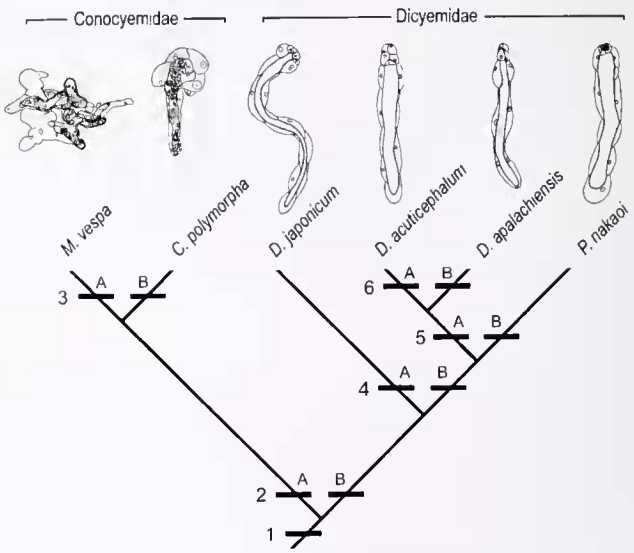

Figure 7. Cladogram of six species of dicyemids based on cell lineages of the vermiform embryos. These dicyemids might have been derived from an ancestor that had a basic cell lineage as shown in Figure 6. Modifications in cell lineages might result in diversity of morphology giving rise to two separate families, namely, Conocyemidae and Dicyemidae. Sketches at top of cladogram indicate the size and shape of the whole bodies of adult stages of each species. Bars represent modifications of the different cell lineages as follows: (1) Early development as shown in Figure 6. (2A) Calotte is formed with a tier of polar cells. (2B) Calotte is formed with two tiers of polar cells; propolars and metapolars present. (3A) Calotte forms a syncytium; diapolars absent. (3B) Calotte is cellular; diapolars present. (4A) Calotte is formed from both $3B^1$- and $3B^2$-cell lineages. (4B) Calotte is formed only from $3B^1$-cell lineage. (5A) Propolars are located perpendicularly above metapolars. (5B) Propolars are obliquely oriented to metapolars. (6A) Cell death occurs both in $3B^1$- and $3B^2$-cell lineages. (6B) Cell death occurs only in $3B^2$-cell lineages; both $4A^1$- and $4A^2$-cells undergo no further divisions.

Table 1

The number of cell divisions in each cell lineage

Dicyemid	Peripheral cell number	A-cell lineage		B-cell lineage	Total cell divisions
		A-cell lineage	a-cell lineage		
Microcyema vespa	10	2	3 (2)	10 (3*)	15 (5)
Conocyema polymorpha	14	6	4 (3)	9 (2)	19 (5)
Dicyema apalachiensis	14	4	3 (2)	11 (2)	18 (4)
Dicyema acuticephalum	16#	6	5 (4)	13 (4)	24 (8)
Dicyema acuticephalum	18#	6	5 (4)	15 (4)	26 (8)
Dicyema japonicum	22#	10	5 (4)	13 (2)	28 (6)
Pseudicyema nakaoi	22	10	4 (3)	13 (2)	27 (5)

The numbers in parentheses represent the number of extremely unequal cell divisions.
* One cell division was not consistently observed.
# From Furuya *et al.* (1994).

cells that do not degenerate. We were able to examine the details of these small cells in several dicyemids, including the species studied by Lameere. In contrast to Lameere's observation, the small cell does not undergo further divisions. In his report, the small cells were exclusively derived from a prospective axial cell (a-cell lineage), but we recognized they are formed in both the a-cell and B-cell lineages, as recognized in the previous study of *D. acuticephalum* and *D. japonicum*.

The small cells eventually die and are eliminated without contributing to the embryogenesis. This is considered to be a programmed cell death as described in the development of infusoriform embryos (Furuya *et al.*, 1992b). In the dicyemids examined, extremely unequal divisions take place four to eight times during embryogenesis (Table 1). The number of such divisions is as definite according to species as the number of peripheral cells. In the a-cell lineage, much programmed cell death appears frequently in dicyemids that consist of a large number of peripheral cells. It seems possible that successive, extremely unequal divisions in the a-cell lineage may be required to maintain an increased amount of cytoplasm in the large axial cell. The axial cell retains most of the cytoplasm of the mother cell and enlarges after each cell division. In most dicyemids, the axial cell elongates as peripheral cell numbers increase. Thus, peripheral cell number appears to be correlated to the number of programmed cell deaths.

The B-cell lineage gives rise to the head region, in which cell death occurs in all dicyemids examined. In contrast, no cell death was observed in the A-cell lineage. The A-cell lineage gives rise to the trunk and tail region, which are composed of standard peripheral cells. Programmed cell death in dicyemids appears in cell lineages associated with remarkably differentiated cells, *e.g.*, the axial cell and calotte cells. Thus, cell death may be intimately involved in the advanced characteristic differentiation of cells.

Several features in developmental pattern and cell lineages among species

The early development of dicyemids is conservative and may be summarized as follows: (1) the first cell division produces prospective cells that generate the anterior peripheral region of the embryo; (2) the second cell division produces prospective cells that generate the posterior peripheral region plus the internal cells within the embryo; (3) in the lineage of prospective internal cells, several divisions ultimately result in the death of one of the daughter cells. Developmental processes to the 7-cell stage are almost identical in the vermiform embryos of the four genera examined (Figs. 5, 6).

In contrast, distinct species-specific differences appear in the order and number of terminal divisions of peripheral cells. Most of the changes in terminal divisions can be correlated with individual body length. Generic differences appear in the number of cells that contribute to the calotte during the final stage of embryogenesis. Distinct morphological features typically emerge following a final cell division or after the embryo escapes from the axial cell of the adult. Subsequent processes, proceeding without cell divisions, are cell differentiation in the head region and cell elongation in the trunk region.

On the basis of cell lineage, a simple cladogram was constructed (Fig. 7). Cell lineages from an agamete to the 7-cell stage were almost identical among species (bar 1). The terminal of B-cell lineage indicates some variation among species. In the family Conocyemidae, a calotte is formed with a tier of polar cells (bar 2A), whereas in the Dicyemidae a calotte consists of two tiers of polar cells, propolars and metapolars (bar 2B). Thus, the tree indicates that two clusters initially separate to form two families. In *Microcyema*, a calotte and peripheral cells form a syncytium (bar 3A), but in *Conocyema* a calotte is cellular and diapolars are present (bar 3B). In *Dicyema japonicum*, the calotte

is formed in $3B^1$- and $3B^2$-cell lineages (bar 4A), but in *D. acuticephalum*, *D. apalachiensis*, and *Pseudicyema nakaoi* the calotte is formed only in $3B^1$-cell lineage (bar 4B). The orientation of propolars to metapolars separates *Pseudicyema* from *Dicyema*. In *Pseudicyema*, propolars are obliquely oriented to metapolars (bar 5B). In *Dicyema*, propolars are located perpendicularly above metapolars (bar 5A). In *D. acuticephalum*, cell death occurs both in $3B^1$- and in $3B^2$-cell lineages (bar 6A), but in *D. apalachiensis* it occurs only in $3B^2$-cell lineage (bar 6B). Based on the above criteria, separation of the dicyemids into two families may be justified; however, the generic state of *Pseudicyema* apparently warrants further study.

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